Annals of Clinical and Analytical Medicine

Original Research

Investigation of the effect of anesthesia type on thiol-disulfide level in cord blood

Cord blood and thiol disulfide levels

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Abstract

Aim: Oxidation and modifications of thiols are considered to be associated with diseases in a way that reveals the cause of some pathological events. It has been demonstrated that changes in analyzed thiol values can be studied as diagnostic or prognostic determinants for various pathologies.

Our study aimed to evaluate the possible changes in thiol-disulfide levels according to the type of anesthesia in patients who underwent cesarean section.

Material and Methods: In the present study, thiol/disulfide levels were measured in the venous blood and cord blood of 41 patients who had general anesthesia and 49 patients who had a cesarean section with spinal anesthesia before and after anesthesia. The correlation between native thiol, total thiol, disulfide, index 1, 2 and 3 levels in the samples collected by the anesthesia method was evaluated.

Results: As a result of the Repeated Measures Analysis of Variance, statistically significant differences were found in the pre- and post-anesthesia venous blood and cord blood measurements of the pregnant women who received both anesthesia in terms of native thiol, total thiol, disulfide, index 1, 2 and 3 variables ($p<0.05$).

Discussion: No significant difference between cord blood thiol/disulfide hemostasis values due to both anesthesia methods was detected. Besides, the difference between the thiol/disulfide pair values in venous blood samples from pregnant women and cord blood samples does not indicate an oxidant status. The results indicate that anesthesia is safe in terms of its potential to cause oxidant damage.

Keywords

Cesarean, General Anesthesia, Spinal Anesthesia, Thiol Disulfide Level, Cord Blood

DOI: 10.4328/ACAM.21346 Received: 2022-08-06 Accepted: 2022-09-12 Published Online: 2022-09-28 Printed: 2022-10-20 Ann Clin Anal Med 2022;13(Suppl. 2):S80-84

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Introduction

The oxidative effects of reactive oxygen species (ROS) are neutralized by the antioxidant capacity of the cells; thus, homeostasis is maintained. Cysteine (Cys) and its disulfide form, cystine (CySS), form the major low molecular weight thiol/disulfide pair in human plasma. Cys/Cyss pool is a critical redox control center in cellular communication [1].

Organic compounds containing a sulfhydryl group in their structure consist of sulfur and hydrogen atoms and are called thiols (SH). The -SH groups of thiols make them sensitive to oxidation, due to these properties, they protect the organism from oxidant damage as they are easily oxidized when they encounter oxidizing agents. Disulfides (-SS-) are a class of dynamic, redox-sensitive covalent bonds formed between two thiol groups [2]. The general thiol pool, composed of the sum of the individual amounts of small compounds such as cysteine, peptides such as glutathione, and thiol proteins such as thioredoxin (Trx) is found at variable oxidation/reduction ratios that are not in equilibrium [3].

Dynamic thiol-disulfide homeostasis (TDH) is an important parameter associated with various biochemical processes, including regulation of protein function, stabilization of protein structure, protection of proteins against irreversible oxidation of cysteine residues, chaperone function, regulation of enzyme functions, and transcription [4].

It is considered that the oxidation and modification numbers of thiols change in a way that reveals the cause of the pathological events existing in some diseases [3]. In fact, the plasma pools of CyS/CySS and GSH/GSSG vary depending on different conditions. It has been demonstrated that the values of redox potentials become more positive (oxidized) with age. GSH/GSSG plasma ratios vary as a result of oxidation at a rate of 0.7 mV/years in all ages between 18 and 93 years [5]. Besides, severe oxidizing thiol/disulfide redox reactions have been shown to arise in many conditions such as chronic alcohol abuse [6], high-dose chemotherapy, type 2 diabetes, and smoking [7]. During the oxidizing reactions, oxygen enters the bond between sulfides in disulfides, causing the bond to break down and the formation of two cysteines called thiols. The thiol oxidation load measured in plasma is often considered to represent plasma oxidative stress resulting from localized or systemic diseases [3]. Thus, increases in measured disulfide values are indicative of an oxidant medium and could potentially be studied as diagnostic or prognostic markers for various pathologies [5, 8].

In the light of all these data, it has been evaluated that there may be a change in thiol/disulfide values due to anesthesia in patients and their babies who are delivered by cesarean section, and that this change may even occur at different rates according to the anesthesia method. This study was designed to reveal the oxidation processes that may occur due to anesthesia or depending on the type of anesthesia and to evaluate the potential of their use as a possible prognostic marker.

Material and Methods

Subjects and collection of blood samples

The study was approved by the Ethics Committee of Yıldırım Beyazıt University, Yenimahalle Education and Research Hospital (Number: 2016/18). The study was conducted by taking cord blood samples of 90 randomly selected pregnant women aged 18-45 who had undergone a cesarean delivery. General anesthesia was applied to 41 of the patients, and spinal anesthesia was applied to 49 of them. Cord blood samples were taken from each pregnant woman in EDTA tubes before and after anesthesia.

Biochemical analyses

A technique in which 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB) namely Ellman's reagent, which takes place as a chromogen, has been used so far to measure thiol levels [9]. Erel and Neselioğlu [10] have modified Ellman's method. This new method evaluates both thiol and disulfide levels in the blood.

In our study, disulfide levels, disulfide/NT, and disulfide/TT ratios, NT/TT levels were analyzed in pregnant venous blood before and after general and spinal anesthesia and in cord blood.

Statistical analysis

The data were analyzed using the SPSS 23 package software. While evaluating the study data, frequencies (number, percentage) were given for categorical variables, and descriptive statistics (mean, standard deviation) were given for numerical variables.

The normality assumption of the numeric variables according to the groups was examined with the Shapiro-Wilk test of normality, and it was seen that they were distributed normally. For this reason, parametric statistical methods were used in the study.

Relationships between the two independent categorical variables were interpreted with the Chi-square analysis. Differences between two independent groups were analyzed with the Independent Sample T-Test. Differences between more than two dependent numerical measurements were checked using Repeated Measures Analysis of Variance. The statistical significance limit was (p value) accepted as 0.05.

Results

As a result of the independent sample t-tests applied, there was no statistically significant difference between the groups that underwent general anesthesia and spinal anesthesia in terms of the variables of gravid gestational week, BMI and birth weight of the baby ($p>0.05$). There was a statistically significant difference in terms of age ($p<0.05$). Accordingly, the mean age in the general anesthesia group was significantly higher than that of the spinal anesthesia group. As a result of the applied chi-square analysis, there was no statistically significant relationship between anesthesia groups and baby gender ($p>0.05$).

As a result of the applied independent sample t-tests, a statistical difference was found between the groups that underwent general anesthesia and spinal anesthesia in terms of hemoglobin and albumin values ($p>0.05$). Before and after both types of anesthesia maternal venous blood NT and cord blood NT; no statistical difference was found in maternal venous blood TT and cord blood TT values before and after both anesthesia types ($p>0.05$). No statistical difference was found in terms of maternal venous blood disulfide levels and cord blood disulfide levels before and after both types of anesthesia ($p>0.05$). No statistically significant difference was

Table 1. Demographic characteristics by groups

	General (n=41)		Spinal (n=49)		t	p
	M	SD	M	SD		
Age (years)	31.71	4.057	29.57	3.808	2.572	0.012*
Gravida	2.78	1.864	2.78	1.476	0.014	0.989
Gestational Week	38.15	1.652	37.82	1.202	1.095	0.277
BMI	24.3	3.276	23.30	3.881	0.955	0.342
Baby's Birth Weight (g)	3302.93	443.876	3283.67	408.879	0.214	0.831
	n	%	n	%	X^2	p
Baby's Gender						
Female	19	46.3	28	57.1	1.044	0.307
Male	22	53.7	21	42.9		

t: Independent Sample T-Test. *:p<0.05

Table 2. Clinical measurements by groups

	General (n=41)		Spinal (n=49)		t	p
	M	SD	M	SD		
Hemoglobin	11.17	1.959	11.41	1.571	-0.632	0.529
Albumin (gr/dl)	4.4	1.125	3.97	1.261	0.272	0.786
Native Thiol						
Before anesthesia [1]	369.05	79.058	349.66	79.362	1.157	0.251
After anesthesia [2]	333.78	90.482	340.95	75.174	-0.411	0.682
Cord blood [3]	390.82	64.988	386.69	70.497	0.287	0.775
F; p	5.926; 0.006*		4.926; 0.011*			
	Difference: 2.3		Difference: 1-3, 2-3			
Total Thiol						
Before anesthesia [1]	402.57	72.201	389.82	74.199	0.822	0.413
After anesthesia [2]	368.38	89.659	381.06	71.611	-0.746	0.458
Cord blood [3]	422.57	63.808	425.01	62.251	-0.183	0.855
F; p	5.568; 0.007*		5.079; 0.010*			
	Difference: 2.3		Difference: 2.3			
Disulfide						
Before anesthesia [1]	21.4	6.963	21.40	6.529	-0.253	0.801
After anesthesia [2]	18.99	7.460	19.94	8.453	-0.562	0.576
Cord blood [3]	15.13	4.720	15.39	5.392	-0.238	0.813
F; p	12.093; 0.000*		12.716; 0.000*			
	Difference: 1-3, 2-3		Difference: 1-3, 2-3			
Index 1						
Before anesthesia[1]	6.61	4.857	6.59	3.292	0.025	0.980
After anesthesia [2]	7.24	7.918	6.46	3.714	0.616	0.540
Cord blood [3]	4.14	2.166	4.33	3.027	-0.329	0.743
F; p	5.862; 0.006*		6.368; 0.004*			
	Difference: 1.3		Difference: 1-3, 2-3			
Index 2						
Before anesthesia [1]	5.61	3.036	5.68	2.427	-0.129	0.897
After anesthesia [2]	5.66	3.940	5.37	2.554	0.420	0.675
Cord blood [3]	3.76	1.627	3.87	2.200	-0.249	0.804
F; p	7.841; 0.001*		7.556; 0.002*			
	Difference: 1-3, 2-3		Difference: 1-3, 2-3			
Index 3						
Before anesthesia [1]	89.00	6.099	88.58	4.869	0.361	0.719
After anesthesia [2]	88.38	7.955	89.00	5.508	-0.440	0.661
Cord blood [3]	92.48	3.254	92.26	4.400	0.250	0.804
F; p	7.842; 0.001*		7.555; 0.002*			
	Difference: 1-3, 2-3		Difference: 1-3, 2-3			

t: Independent Sample T-Test; F: Repeated Measures Analysis of Variance *:p<0.05

found in terms of maternal venous blood index 1 values and cord blood index 1 values before and after both anesthesia types (p>0.05). No statistically significant difference was found in terms of maternal venous blood index 2 values and cord blood index 2 values before and after both anesthesia types. Lastly, no statistically significant difference was found in terms of maternal venous blood index 3 values and cord blood index 3 values before and after both types of anesthesia (p>0.05).

As a result of the applied Repeated Measures Analysis of Variance, statistically significant differences were found between the venous blood and cord blood measurements of the mothers who received general anesthesia before and after anesthesia in terms of NT. TT disulfide index 1, index 2, and

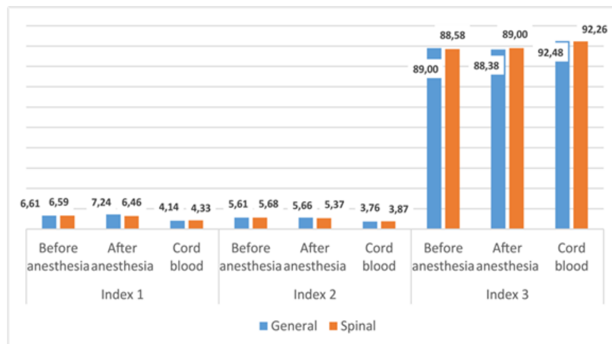

Figure 1. Native thiol and total thiol values in venous blood and cord blood samples before and after anesthesia in pregnant women who received general and spinal anesthesia

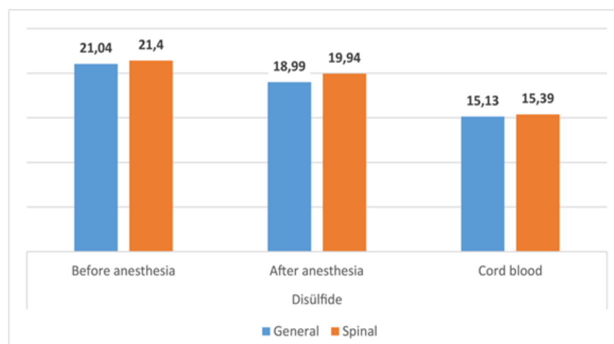

Figure 2. Disulfide values in venous blood and cord blood samples before and after anesthesia in pregnant women who received general and spinal anesthesia

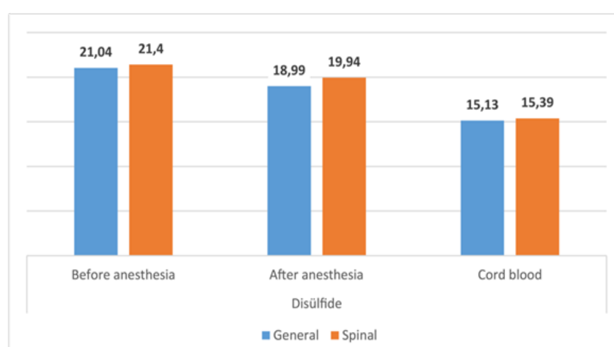

Figure 3. Index 1, 2 and 3 values in venous blood and cord blood samples before and after anesthesia in pregnant women who received general and spinal anesthesia

index 3 variables (p<0.05). Cord blood NT levels in the general anesthesia group were significantly higher than venous NT levels after anesthesia. Cord blood TT levels in the general anesthesia group were significantly higher than venous TT levels after anesthesia. Cord blood disulfide levels were significantly lower than venous disulfide levels before and after anesthesia in the general anesthesia group. Cord blood index 1 levels were significantly lower than pre-anesthesia venous index 1 levels in the general anesthesia group. Cord blood index 2 levels were significantly lower than venous index 2 levels before and after anesthesia in the general anesthesia group. Cord blood index 3 levels were significantly higher than venous index 3 levels before and after anesthesia in the general anesthesia group.

As a result of Repeated Measures Analysis of Variance, statistically significant differences were found in the spinal anesthesia group in terms of NT, TT, disulfide, index 1,index 2, and index 3 variables in the venous blood and cord blood samples before and after anesthesia (p<0.05). Accordingly cord blood NT levels were significantly higher than venous blood NT levels before and after anesthesia in the spinal anesthesia group. Cord blood TT levels in spinal anesthesia group were significantly higher than venous blood TT levels after anesthesia. Cord blood disulfide levels were significantly lower than venous blood disulfide levels before and after anesthesia in the spinal anesthesia group. Cord blood index 1 levels were significantly lower than venous blood index 1 levels before and after anesthesia in the spinal anesthesia group. Cord blood index 2 levels were significantly lower than venous blood index 2 levels before and after anesthesia in the spinal anesthesia group. Cord blood index 3 levels were significantly higher than venous blood index 3 levels before and after anesthesia in the spinal anesthesia groups (Figures 1, 2, and 3).

Discussion

One of the main purposes of many scientific studies performed is to reveal the markers that will show the prognosis of the diseases and will serve as diagnostic criteria. Likewise, a significant number of studies have been conducted on the possible association of various pathological conditions and their use as a potential oxidative marker by analysis of thiol and disulfide molecules [11, 12]. In this study we examined whether there were significant changes in thiol disulfide values in the venous blood and cord blood of patients who delivered by cesarean section depending upon anesthesia. In fact, we formed our study groups in this direction considering that this change may occur at different rates according to the anesthesia method. We aimed to reveal the oxidation processes that may occur due to anesthesia or depending on the type of anesthesia and to evaluate their use as potential prognostic markers.

During the oxidizing reactions that occur due to the formation of free radicals in tissues. A disulfide molecule is formed from two thiol (cysteine) molecules by establishing bonds between thiol molecules. The amount of thiol molecules after oxidation decreases after conversion of disulfide. The change in favor of an increase in the amount of disulfide in the thiol/disulfide redox couple is an indication of the presence of free oxygen radicals in the medium and that antioxidant defense works against them [3].

Scientific studies have shown that the thiol/disulfide pool changes in favor of disulfide due to oxidation damage in many pathological conditions [13]. In our study, we found that cord blood NT and TT values of pregnant women were higher when compared to venous blood values in both anesthesia groups. On the contrary, we found that the disulfide values decreased. When cord blood is taken as a reference, in the first place, maternal venous blood is used with high disulfide ratios, which is significant in terms of oxidation indicating that pregnant women are affected by anesthesia. However, venous blood values collected from pregnant women were collected both before and after anesthesia. Therefore, the fact that there was no significant difference between the values of pregnant venous blood taken after anesthesia and pregnant venous blood taken after anesthesia indicates that pregnant women did not suffer any oxidant damage due to anesthesia. However, NT and TT values in the cord blood of the baby, which was in a separate compartment from the maternal venous blood due to the placental barrier, were statistically higher than the NT and TT values in the maternal venous blood. This significant increase in cord blood samples, which is an example of post-anesthesia indicates that the baby was not exposed to an oxidant reaction due to anesthesia. This situation was found in the same way in both types of anesthesia (general and spinal anesthesia). Since the conversion of thiol groups to disulfides as a result of oxidant reactions requires a decrease in thiols, disulfides increase like a seesaw. However, the results of the study showed the opposite change. In parallel with this, the fact that index 1 values, which is the ratio of disulfide values to NT values and index 2 values, which is the ratio of disulfide values to TT values are found to be significantly decreased, indicates that the amounts of NT and TT do not participate in the formation of disulfide, that is to say they do not undergo oxidation. Besides index 3 values, which is the ratio of NT values to TT values, were found to be significantly increased. This showed that native thiols, which are sensitive to oxidation maintain their position in the total thiol pool. In previous studies, thiol values were found to be significantly higher than disulfide values according to cord blood measurements in cesarean deliveries [14]. These results contradict our results. However, the results in this contradicting study were evaluated not in terms of anesthesia and type of anesthesia but in terms of normal or cesarean delivery. In another study, postoperative cord blood samples from pregnant women who delivered by cesarean section were evaluated in terms of general and spinal anesthesia [15]. In this study, it was shown that the cord blood oxidation levels of pregnant women who received general anesthesia were higher than those who received spinal anesthesia based on increased disulfide values in cord blood samples from pregnant women. The results of this research also contradict the results of our research. However, since the results obtained in this study were obtained only between two types of anesthesia, this does not clearly prove that the results obtained are actually the increased oxidant level due to anesthesia because there was no reference cord blood values obtained from pregnant women who had a normal delivery without anesthesia in the study, and the evaluation was made only between anesthesia groups. If the cord blood values of pregnant women who had a normal delivery could be checked, the effects of anesthesia could be fully revealed according to the values to be obtained and we would be able to decide whether this contradicts our study or not.

On the other hand, the fact that babies are protected from possible oxidant stress despite the anesthesia applied to pregnant women can be attributed to the placental barrier. However, studies including cord blood thiol/disulfide analyzes have shown that infants suffer oxidant damage in pathologies belong to their mother [16]. This makes us think that the oxidation parameters within the normal limits in the cord blood we found in our study are not a result of the placental barrier.

Another result we obtained in our study is that the NT and TT levels in the venous blood of pregnant women are lower than the cord blood values representing the infants' blood. The NT and TT values in an elderly mother are lower than the values of the baby who is at the beginning of his/her life and this indicates a decrease due to oxidative events experienced over the years. The results of our study have parallelism with the studies showing that the thiol/disulfide pool decreases with each year of life with age [5, 17].

Conclusion

When oxidant status was evaluated in terms of thiol/disulfide couples in the study, it has been demonstrated that general and spinal anesthesia applications did not create oxidative stress in both pregnant women and babies. This indicates that both types of anesthesia are safe methods for babies including pregnant women in terms of oxidant damage. On the other hand, a similar study can be planned so that an evaluation in this direction covers all redox mechanisms. Thus, the oxidant results of the anesthesia used for cesarean section in pregnant women and especially babies will be clearer and more precise.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: *None*

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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How to cite this article:
Ozgul Ozgan Celikel, Gamze Gok, Ozcan Erel, Filiz Yildirim. *Investigation of the effect of anesthesia type on thiol-disulfide level in cord blood. Ann Clin Anal Med 2022;13(Suppl. 2):S80-84*